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Health Effects of Benzene A Review

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A Review

Health Effects Of Benzene

Committee on Toxicology

Assembly of Life Sciences

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Committee on Toxicology
Assembly of Life Sciences
National Research Council

National Academy of Sciences

Washington, D.C.

June 1976

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The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the Committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

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HEALTH EFFECTS OF BENZENE: A REVIEW

SUMMARY

Acute exposure to benzene produces toxic effects on the central nervous system; however, in order to evaluate the chronic effects, consideration must be given to the myelotoxic and possible chromosome-damaging and leukemogenic effects of benzene. The time required for expression of chronic benzene toxicity indicates a vast difference in individual sensitivity. Some workers have shown signs of benzene poisoning after brief chronic exposures while others display resistance for long periods of time. The myelotoxic effects of benzene poisoning have been documented in the shoe and rotogravure industries in the United States, Turkey, Italy, and France. Aplastic anemia associated with benzene exposure may well represent the most significant hazard.

Benzene has long been suggested as a leukemogenic chemical based on many individual cases of leukemia which have been linked to benzene. Although essentially all these cases were subjected to mixed exposures, benzene was the chemical common to all cases. The first extensive epidemiological survey to demonstrate a correlation between leukemia and benzene exposure was reported by Aksoy et al.[4] in 1974. The study of 28,500 shoeworkers showed an annual incidence of leukemia of 13/100,000 compared to 6/100,000 in the general population. Thorpe's epidemiologic study[61] on 38,000 petroleum workers who had potential exposures to benzene failed to indicate an increased incidence of leukemia. In spite of the inherent shortcomings of Thorpe's study, it does seem to indicate that there is not an outstandingly high incidence of leukemia in the workers studied. His negative data, in contrast to Aksoy's findings, may be due to the inadequacy of the follow-up techniques, lack of sufficient exposure to benzene due to good work conditions, relatively weak or no leukemogenic activity of benzene, or the absence of some necessary cofactors.

Most cases of severe benzene intoxication have been reported in workers exposed to rather high concentrations of benzene under somewhat unhygienic working conditions. It is probable that all cases reported as "leukemia associated with benzene exposure" have resulted from exposure to rather high concentrations of benzene and other chemicals.

It has been suggested in the literature that "benzene-induced leukemia" may occur only in individuals who are highly sensitive because of genetic constitution or because of synergistic action of other chemical or physical environmental agents. A co-leukemogenic role for benzene would explain the failure to induce leukemia in benzene-exposed animals.

The state of the benzene literature makes it very difficult or impossible to reach a firm conclusion on the dose-response relationship in chronic exposure of humans to benzene. The details of the extent of exposures are either inadequate or absent. Even in cases where some concentrations of benzene are reported, the stated concentrations were based on occasional measurements of short durations. The role of benzene

metabolism in its toxicity and the significance of benzene-induced chromosome aberrations are currently unclear. It appears that a metabolite of benzene may be responsible for its myelotoxic effects.

Bases on available literature, it can be concluded that benzene may be associated with leukemia; therefore, benzene must be considered as a suspect leukemogen. More definitive data are required for an accurate assessment of the myelotoxic, leukemogenic and chromosome-damaging effects of benzene.

RECOMMENDED RESEARCH

The review of the benzene literature clearly indicates that more definitive data are required to assess accurately the human health effects of benzene. Some areas of research which would be expected to yield scientific results helping to elucidate the mechanism of chronic benzene effects are listed below:

1. Inhalation studies using multiple animal species to provide a firm data base for dose-response relationship to myelotoxicity, and to determine leukemogenic potential. Another approach would be to expose leukemia-prone mice to benzene.

2. Studies to assess the co-leukemogenic potential of benzene. One possible approach would be the use of a combination of benzene and radiation exposures with the necessary controls.

3. More detailed metabolic studies to determine the animal model most representative of humans, and to study the metabolism of benzene in bone marrow. Since most carcinogens bind covalently to cellular nucleic acid in the target tissue, 14C- or ^{3}H-labelled benzene could be used to investigate this possibility in experimental animals or human tissues in vitro.

4. Studies to provide substantiating data on concentration and dose/effect relationships between the severity of hemopathy and frequency and significance of chromosomal abnormalities.

5. Determination of end-points for early detection of reversible and irreversible changes due to chronic benzene exposure including the possibility that benzene exposure results in specific qualitative and quantitative changes in blood elements.

6. Further epidemiological studies are needed, both retrospective and prospective, particularly among industrial workers exposed to benzene and related solvents. The possibility that leukemogenesis involves mixed exposure (benzene plus another agent or factor) should be stressed in the design of these studies.

INTRODUCTION

This report is in response to EPA's request for compilation, summarization, and evaluation of the existing toxicological and epidemiological information relevant to public exposure to benzene. Particular emphasis will be devoted to the effects on hematopoietic organs and the existing evidence relating benzene to leukemia.

Browning[12] wrote an extensive review article on benzene toxicity which was published in 1965. A number of recent review articles[8,9,53,60,65] have appeared, including the National Institute for Occupational Safety and Health (NIOSH) criteria document, "Occupational Exposure to Benzene"[65] and a similar German document, "Benzene in the Work Environment."[8] These review articles and selected research papers, especially recent ones, will provide the resources for this report. In addition to the extensive collection of benzene literature in the library of the Advisory Center on Toxicology, Medline searches were utilized to survey the current literature on benzene.

Benzene was discovered by Faraday in 1825 and has become an important industrial chemical. The development of the chemical industry, especially in the field of plastics, has firmly established the necessity for large quantities of benzene as the starting material for chemical synthesis. Benzene is consumed by the U. S. chemical industry at the rate of 1.4 billion gallons annually and is expected to increase when additional production facilities become available. It has been estimated by NIOSH that approximately 2,000,000 workers in the United States have potential exposure to benzene. Additional uses for benzene are continually being found such as the possible use of benzene and other chemicals to replace lead compounds as antiknock components in motor fuels. Public exposures such as the presence of benzene in gasoline and the possibility of increased content in gasoline causes increased concern for benzene as a significant environmental pollutant.

GENERAL PROPERTIES AND USES

Benzene (C_6H_6) is the parent hydrocarbon of the aromatic group, the resonant cyclic compounds containing only carbon and hydrogen. Commercial benzene is sometimes called "benzol." At room temperature benzene is a colorless, clear liquid with a density of 0.87 g/cm^3 and boiling point of 80.4 C. It has a melting point of 5.4-5.5 C, a vapor pressure of 74.6 mm at 20 C and a flash point of 12 C. Benzene is slightly soluble in water and miscible with alcohol, chloroform, ether, carbon disulfide, acetone, glacial acetic acid and carbon tetrachloride. The high volatility of benzene at room temperature is a very useful characteristic, but also the chief source of hazard to workers.

Benzene is produced chiefly from coal tar distillation; however, it is also produced from petroleum by catalytic reforming of light naphthas from which it is isolated by distillation or solvent extraction. Industrial uses of benzene have included the following: extraction and rectification, preparation of benzene derivatives for the chemical and pharmaceu-

tical industries, as a solvent in the rubber industry, in glue for the footwear industry, the preparation and use of inks in the rotogravure industry, as a thinner for paints, as a degreasing and cleaning agent, and as an anti-knock fuel additive. The industrial processes involving production of benzene and chemical synthesis usually take place in sealed and protected systems. The uses of benzene as a solvent and cleaning agent have been decreased significantly, usually by using less toxic substitutes.

Benzene in Gasoline

Since a vast amount of literature continues to accumulate on the chronic effects of benzene exposure, the benzene content and effect of benzene exposure from gasoline have become the subject for numerous studies.

Parkinson[47] has conducted an investigation of working conditions at typical filling stations and at some oil company bulk loading installations in England. The benzene concentration in the atmosphere measured at three sites under variable weather conditions ranged from 0.3-3.2 ppm for gasoline containing benzene in concentrations ranging from 3.1 to 5.8% (V/V). At bulk loading facilities with benzene enriched gasoline up to 33% (V/V), the results were 1.4-9.4 ppm. The results obtained in this study led Parkinson to conclude that only trivial exposure to airborne benzene vapor occurred when gasoline with normal benzene content up to 5% (V/V) was dispensed at typical filling stations.

In 1972, Sherwood[58] reported results from a petroleum loading installation in Italy. The benzene content was approximately 2.7% by weight of normal petrol and 4.5% for premium petrol. Loaders were exposed to mean concentrations of 1.6-2.5 ppm over a 5-hour period; however, the weigher working between the tracks was exposed to a mean concentration of 20 ppm. At the end of the work day the phenol content of the urine for the workers correlated well with the exposure. The following morning the phenol was normal in the loaders' urine, but remained elevated for the weigher.

Berlin et al.[9] have recently published a review concerned with the likely consequence of an increase in the aromatic content of motor fuels. Due to the limitations of the European refining industry and the requirement to reduce lead, they estimate that the aromatic content can be expected to increase to approximately 48-50%, since it takes above a 2% increase in the aromatic content to improve the octane rating by one unit.

Although the actual exhaust composition will depend on the type engine and combustion conditions, some generalizations can be made regarding the effects of increased aromatic content of fuel on the exhaust emissions. The total volume of hydrocarbon emission decreases slightly and the photochemical reactivity increases slightly; however, this is considered to be of only secondary importance in Sweden.[9] Oxidants were not increased. Although not conclusive, results indicate a trend toward increased aerosol formation with increasing aromatic content. The total

amount of aldehydes is decreased and the amount of potentially carcinogenic compounds (polynuclear aromatic hydrocarbons, PNA) will increase with an increased aromatic content, but this will be small when compared to other variables that affect emission of PNA.

Runion[52] has published a study on the benzene content of Gulf's gasolines in both the liquid and vapor phases, demonstrating a straight-line relationship between benzene volume percent levels in the two phases; however, it was observed that the volume percent in the vapor phase is less than one-half the volume percent in the liquid phase. The volume percent benzene in the gasoline vapors increased during evaporation of a typical gasoline, but would not be significant in most cases, as it is estimated that gasoline available at the typical service station pump has not evaporated more than 1%. Runion concluded that it would be difficult to exceed the Threshold Limit Value (TLV) of 10 ppm for benzene[7,62] from exposures to gasolines containing 5% or less benzene. In 1972, Swiss authorities enacted legislation limiting the benzene in gasoline to a maximum of 5% by volume.

ACUTE TOXICITY

Benzene exposure causes acute toxic effects on the central nervous system. Exposure to massive concentrations, in the region of 2.5% by volume in air, is rapidly fatal. The symptoms are those of central nervous system depression which may be preceded by convulsion, and death usually follows from cardiovascular collapse. Browning[12] has reported that fatal cases recorded have usually occurred when benzene was inhaled in closed spaces such as tanks. Severe non-fatal cases exhibit similar symptoms, but recover after a period of unconsciousness. Milder exposures show euphoria followed by giddiness, headache, nausea, and staggering gait, and unconsciousness if exposure continues.

The severity of the symptoms of acute benzene toxicity and the possibility of fatal outcome depend primarily on the concentration and duration of exposure, but it is clear from documented fatal cases that marked variations exist in individual susceptibility. This is suggested by the fact that rescuers of workmen lying unconscious in tanks have died while the original victim survived. Some authorities have interpreted these data as an implication of the adrenals in benzene poisoning; however, it could be due to cardiac sensitization with resultant fatal arrhythmias. The chief anatomical changes determined during post-mortem examinations of cases of acute fatal benzene poisoning are the presence of petechial hemorrhages and abnormal fluidity of the blood.

CHRONIC TOXICITY

Benzene Induced Blood Disorders

In the earliest reports of benzene toxicity[56], benzene was recognized as a powerful leucotoxin, destroying white cells of the circulating blood, and severely injuring the bone marrow. Based on clinical records and ex-

perimental work, the features of chronic benzene poisoning appear to be marked changes in the blood secondary to selective localization and accumulation of the chemical in the hemopoietic tissue.[53] In chronic benzene poisoning, changes in other organs and tissues are secondary to changes in blood parameters and usually depend on these changes.

The signs of chronic benzene poisoning can appear any time from a few weeks to several years of exposure.[53] Typical symptoms may be headache, a slight dizziness, nausea, vertigo at the end of work day, stomach pain, loss of appetite, or feeling cold. If intoxication is more severe, the clinical signs are more pronounced, such as weakness, blurring of vision and dyspnea on exertion. The mucous membranes and skin may appear pale. There is evidence of a hemorrhagic tendency which may consist of petechia, easy bruising, epistaxis, bleeding from the gums or menorrhagia. Under these conditions blood examination usually suggests the presence of marrow hypoplasia. This disorder is accompanied by a decrease in red blood cells in the peripheral blood with a parallel or slightly lesser fall in the hemoglobin level, indicating that it is a normochromic or mildly hyperchromic form of anemia. Macrocytosis may occur as is often the case with hypoplastic anemia. Regeneration appears to be slight or absent, the number of white cells is low, usually less than 4000 per mm^3 and there is a decrease in neutrophils and a relative lymphocytosis. In addition, there is a shift to the right in the differential count, the platelets are reduced in number and their morphology is altered (anisocytosis, staining defects, pyknosis of the chromomere). The hemoglobin turnover is reduced; the serum bilirubin is low and the amount of urobiliogen reduced, if no concomitant hepatic lesions are present. Red cell fragility remains normal but there is a rise in serum iron due to defective utilization of this element. The possible mechanism of this effect will be discussed later. The marrow usually shows a diminution of cells, including absence of megakaryocytes, but sometimes the most immature forms of red and white cells are present in large numbers. Relatively few are intermediate or late normoblasts, myelocytes or metamyelocytes because of the marked shift in maturation.

The available evidence does not indicate that the reported benzene-induced blood dyscrasias differ in any way from similar dyscrasias which are caused by other myelotoxic agents or for which the etiology is unknown.

The course of hypoplastic marrow effects associated with benzene exposure may be progressive unless action is taken to remove the person from the toxic agent and to administer appropriate treatment.[53] If this is accomplished in time, complete recovery may follow, but the process is slow and defects in the blood constituents may continue for several years, particularly with the white cells. With repeated exposures to benzene, recurrences may readily appear and be more severe than previously. The condition may become irreversible with or without repeated exposure, and may develop into a state of chronic progressive pancytopenia; or it may develop into an acute or subacute disease such as acute pancytopenia or leukemia.

Chronic pancytopenia has been associated with intense and prolonged exposure to benzene or as a result of special characteristics relating to the individual[53]. Without an exposure to benzene for two years, the appearance of the severe blood disorders is rare. The clinical picture is similar to that previously described, but the pallor and the anemia are more noticeable. The hemoglobin level may be as low as 20% and the red cell count may be 1 million per mm^3 or less. The leukocyte count falls to less than 1000 per mm^3, the neutrophil polymorphs being as low as 1 to 2%, and the platelets are barely detectable. The bone marrow becomes almost acellular. Hemorrhages are widespread, occurring mainly from severe thrombocytopenia, but occasionally from hypoprothrombinemia and hypofibrinogenemia.

Acute pancytopenia has been associated with benzene exposure[53]. The onset may be abrupt, even in subjects whose blood picture has remained satisfactory or at least not been severely impaired by benzene. There is severe pyrexia with toxemia and a rapid and severe drop in the level of all the circulating elements of the blood. Anemia is usually hyperchromic and macrocytic and hemorrhages are severe and widespread in these cases. Blood cultures may prove positive from streptococci and sometimes for staphylococci or E.coli. The outcome is fatal and death occurs in a few days or weeks; however, modern therapeutic methods may increase survival time and bring about temporary remissions.

Aksoy et al.[1] conducted a study on 217 workers who had been exposed to benzene in the workplace at concentrations ranging between 30-210 ppm from 3 mo to 17 yr. When compared to 100 control subjects, 51% of the workers, showed hematological abnormalities attributable to chronic benzene exposure. Leukopenia was detected in 9.7% of the workers; thrombocytopenia in 1.84%. Leukopenia associated with thrombocytopenia was found in 4.6% and leukopenia as a part of pancytopenia in 2.76% of the cases. Thus 17.5% of the workers had a white cell count of less than $4000/mm^3$. Hemoglobin levels were less than 12 g/100 ml in 33% of the workers and in 32.7% the packed cell volume was less than 40%. While a mild or moderate hypochromic or normochromic anemia was present in 33% of the workers, the same result was found in 21% of the controls. Oral iron was given to some of the anemic workers with a resulting disappearance of the anemia; therefore, the presence of an iron-deficiency anemia simultaneously with chronic benzene poisoning was a possibility.

Later Aksoy et al.[3] examined blood changes in workers suffering from pancytopenia associated with long-term exposure to benzene. The estimated exposure level varied from 150-650 ppm for 4 months to 15 years. Clinical features and hematological data for all patients were generally similar; however, the bone marrow results were different. It was found that the patients could be classified into four groups. In addition to pancytopenia the following findings were exhibited: hypoplastic or acellular bone marrow, hyperplastic bone marrow, normoblastic bone marrow, and large erythroid precursors, possibly a preleukemia state. Clinical symptoms included macrocytic anemia, mild reticulocytosis, hyperbilirubinemia, erythroblastemia, and increase in quantitative osmotic fragility and elevated lactate dehydrogenase levels. The fetal hemoglobin (HbF) content

in 20 out of 24 patients was increased although the mean values in groups with hypoplastic, hyperplastic and normoblastic bone marrow were essentially identical. The hemoglobin A_2 (HbA_2) level was normal for 21 of 24 pancytopenia patients. Two showed a slight decrease and one a definite decrease. The mean age of the group with normoblastic bone marrow had the lowest mean age. The illness was less severe and the outcome more favorable in this group; however, there was no significant decrease in duration of exposure, suggesting that older patients were more severely affected by chronic exposure to benzene.

Lange et al.[37] has investigated the immunoglobulin levels in the sera of 35 workers occupationally exposed to a combination of benzene, toluene and xylene at estimated concentrations of 0.11 - 0.158 mg/l, .203 - .27 mg/l and .224 - .326 mg/l, respectively. The levels of three classes of immunoglobulins (IgG, IgA, and IgM) of these workers were compared to those of a control group consisting of 42 healthy adults. The IgG and IgA levels decreased significantly, while the IgM level increased. These results were found regardless of the period of exposure to the solvents. It is suggested that the fall in IgG may reflect a suppressive action of benzene on immunoglobulin-producing cells, resulting in the inhibition of DNA synthesis and thus giving rise to IgM level due to impaired feedback control of IgM synthesis by the lower level of IgG. This study is treated as only benzene exposure disregarding possible effects of toluene and xylene.

Since 10 workers in the above study were found to harbor autoleukocyte agglutinins, Lange et al.[38] performed a leukoagglutination test and a cytotoxic test with leukocytes on 76 workers occupationally exposed to the three solvents (benzene, toluene and xylene) and results compared to a control group. Only one case out of 41 controls exhibited the presence of leukocyte agglutinins; the agglutinins reacted with autoleukocytes but failed to produce agglutination of leukocytes from "O" Rh-negative donors. In contrast, 10 exposed workers out of 35 had leukocyte agglutinins reacting with autoleukocytes from "O" Rh-negative blood donors. None of the sera was positive in the Gorer and O'Gorman cytotoxic test. Data from this study suggest the occurrence of allergic blood dyscrasia in some people exposed to benzene and its homologues.

A further study conducted by Smolik et al.[59] on workers exposed to benzene, toluene and xylene showed that 62 of 79 exposed subjects had a complement level lower than the mean value of the control group. The mean value of the exposed group was statistically significantly lower than that of the controls ($p<0.01$). The data of this study are consistent with the previous reports and suggest the involvement of immunologic factors in the mechanism of the effects chronic exposure to benzene, toluene and xylene.

Yakushevich in Russia[68] reported the results of a study on the dynamics of the leucocyte content in the blood of white rats exposed to benzene (from 50-5000 mg/m^3), and toluene (from 30-1000 mg/m^3). Under conditions of chronic inhalation, leukopenia developed under the influence of benzene or toluene while leukocytosis occurred under the influence of xylene. The

degree of leukopenia caused by benzene or toluene was a function of concentration and the duration of the inhalation exposure. The time of onset of a significant change in the quantity of leukocytes increased as the concentration of the chemical under study decreased. Blood cholinesterase activity during chronic inhalation of benzene, toluene or xylene decreased as a function of their concentration and the duration of exposure. The decrease in activity of the enzyme occurred at later times as the concentration of the chemical decreased.

Kissling and Speck[34] have reported results on studies of the mechanism of benzene-induced aplastic anemia. Rabbits receiving a subcutaneous dose of 0.2 ml/kg/d of pure benzene demonstrated peripheral pancytopenia within 6-12 weeks. A marked decrease of leukocyte, thrombocyte and reticulocyte counts occurred within 3 weeks which was followed by a drop of the erythrocyte count and hemoglobin concentration. A left shift in erythropoiesis and myelopoiesis was observed in the bone marrow two weeks after the beginning of benzene injections. Autoradiographic studies on the bone marrow cells of rabbits with benzene-induced pancytopenia showed a marked interference with DNA and RNA synthesis. It was suggested by Kissling and Speck that the pancytopenia which follows benzene exposure may be due to disturbed DNA- and RNA-synthesis rather than a failure of the hemopoietic stem cell.

Chromosomal Effects

In 1964 Pollini and Colombi[49] reported lymphocyte chromosome damage in patients with benzene-induced aplastic anemia. Abnormal karyotype and deletion of chromosomal material were frequently encountered. This report was soon followed by another study of Pollini et al.[50] which attempted to relate chromosomal alterations to the severity of benzene-induced blood dyscrasia. The karyotypes of marrow parenchyma cells and peripheral blood lymphocytic cells were examined in 4 subjects with temporary or progressive blood dyscrasias. The incidence of heteroploid chromosomal patterns ranged around 70% in the blood and marrow of each subject; however, there was no correlation to the degree of evolution of blood dyscrasia. Likewise the dispersion of chromosome number in the cells examined failed to relate to the severity of the condition. Reports on numerous studies[20,21,27,32,57,63] involving benzene-induced hemopathies have yielded similar changes.

Vigliani and Forni[66] reported chromosomal aberrations of both the stable and unstable type. In general the chromosomal aberrations were higher in peripheral blood lymphocytes of workers exposed to benzene than in those of controls. This was true even in the absence of overt signs of bone marrow damage. The stable type was found to persist several years after recovery from benzene hemopathy. Toluene and xylene failed to cause a significant increase in chromosomal aberrations. The hypothesis was suggested that benzene might induce various types of chromosomal aberrations and that leukemia may develop in cases when potentially leukemic clone with selective advantage is produced as a toxic response to benzene exposure.

Two of the more recent articles from the same clinic in Italy,

Clinica del Lavoro "Luigi Devoto," Milan[22,19] present the most systematic studies that have been conducted. In the first, 34 workers in a rotogravure plant and 34 matched controls were compared. Because of the large number of benzene hemopathies, as previously reported in this and other industries, the government banned the use of benzene in certain industries and imposed a Maximum Allowable Concentration (MAC) of 25 ppm in 1953. Therefore, this study was complicated by the fact that only 10 workers were exposed to benzene (at levels of 125-532 ppm measured only in April 1953), all well above the MAC from employment to this date (from less than 1 yr to 22 yrs). In 1953 benzene was replaced with toluene containing traces of xylene. The concentration of toluene in various locations in the plant ranged from 0-240 ppm from 1954-1956; during 1957-1967, the concentrations were 56-824 ppm. The remaining 24 workers were exposed to the toluene only. The matched controls were exposed to neither of the solvents. The 10 subjects exposed to benzene (and toluene) exhibited 1.66% and 0.62% C_u cells and C_s cells, respectively. Toluene exposed subjects had 0.80% and 0.08% C_u and C_s cells, respectively, which is not significantly different from the control subjects with 0.61 C_u cells and 0.09% C_s cells. Each metaphase was classified as follows: A, apparently normal; B, with chromatic aberrations; C_u, with unstable chromosome changes (fragments, rings, or dicentric chromosomes); or C_s, with stable chromosome aberrations (abnormal monocentric chromosomes due to deletions, translocations, trisomies, inversions, etc.). In C_u cells the presence of a fragment was considered as one break, the presence of a dicentric ring chromosome as two breaks. The unstable and stable chromosomal aberrations were significantly higher in the benzene group compared with controls ($P<0.01$) and the toluene group ($P<0.05$). A slightly higher rate of cells with more than 46 chromosomes, primarily due to a higher rate of polyploid cells, was observed in the benzene group; however, the difference was not statistically significant. No significant differences were found between chromosomal changes in the toluene group and the control group.

In the later study from Milan,[19] 25 subjects, who had recovered from benzene hemopathy (4 with bone marrow pathology and in 3 subjects who had recovered from acute exposure), revealed significant increased ratios of stable and unstable chromosome aberrations, which in most cases persisted for several years after cessation of exposure and recovery. Abnormal metaphases in subjects recovered from benzene hemopathy were 1.89% C_u and 1.22% C_s which compares with 0.49% C_u and 0.04% C_s for the controls. At the time of these chromosome studies, the hematologic follow-up gave normal blood counts in most cases; a few subjects still had a moderate anemia and one still showed anemia, leukopenia and moderate thrombocytopenia. One woman was eight months pregnant when admitted to the clinic in 1968 with pancytopenia after occupational exposure to benzene. Although severely pancytopenic, the patient delivered an apparently normal boy. The following year the patient had another pregnancy and delivered a normal baby girl. A cytogenetic study performed on the peripheral blood of the newborn boy did not indicate any chromosomal abnormalities.

A study by Tough *et al.*[64] has shown a correlation between age and chromosomal aberrations as well as between benzene exposure and chromosome aberrations. No correlation was found between the number of C_u cells

and the years of exposure to benzene in any of the groups studied.

This series of studies indicated no correlation between the persistence of chromosomal changes and the degree of benzene poisoning. It is significant to note that no quantitative data on total benzene exposure were available on all of the above studies on chromosome aberration on humans. All indications point to very high levels probably several hundred ppm.

Koizumi et al.[35] has reported a decreased DNA synthesis by cultured human leukocytes and by HeLa cells that was observed from 2.2×10^{-3} M benzene. Chromosome aberrations consisting of breaks and gaps were observed in cultured human leukocytes in dose levels of benzene of both 1.1×10^{-3} M and 2.2×10^{-3} M; however, the significance of this finding for human exposures at low concentrations must be questioned.

Kissling and Speck[33] studied the chromosomal aberrations in rabbits injected subcutaneously with a dose of 0.2 mg/kg/d of pure benzene. The karyotype was normal in 15 of 16 test animals; however, the frequency of mitoses with chromosomal aberrations was initially in the range of 5.9%; but increased to 57.8% after an average of 18 weeks.

Dobrokhotov of Russia[16] has reported studies using rats exposed to 0.2 g/kg/d benzene, 0.8 g/kg/d toluene and a mixture of benzene and toluene at doses of 0.2 and 0.8 g/kg/d respectively. Chromosomal aberrations were similar at the above administered doses of benzene and toluene, thus indicating benzene as a stronger mutagen. The combined action of benzene and toluene on the white rat took the form of total summation of the evoked genetic effect of the two chemicals given separately.

Pierre[48] has reported interim results of a study attempting to determine the value of chromosomal analysis in the diagnosis of preleukemia. Cytogenetic abnormalities were found in the preleukemic phase of acute leukemia at approximately the same frequency and type exhibited in the acute leukemia. Pierre stated that the failure to demonstrate an abnormal cell line would not exclude preleukemia as it may be evolving into a leukemia not characterized by observable cytogenetic abnormalities. Sandberg[55] has determined that visible chromosomal aberrations do not occur in half the cases of acute leukemia and in some cases of chronic myelocytic leukemia and almost all cases of chronic lymphocytic leukemia.

BENZENE EXPOSURE AND LEUKEMIA

The review of the literature reveals numerous cases of leukemia attributed to benzene exposure; however, in essentially all reported cases, benzene was only one of the chemical agents to which the worker was exposed. For example, in the shoe industry, benzene was a solvent in glue and in the rotogravure industry, benzene was a solvent for ink. Yet articles have titles such as "Acute leukemia due to chronic exposure to benzene."[2] This paper describes four cases of acute leukemia which developed in shoemakers using benzene-containing adhesives. It is important

to emphasize that the following discussion relating to human exposure to benzene actually involve mixed exposure to chemicals with benzene being a common one.

In 1961 Cronkite[13] made the following statements:

> The heart of the problem in the induction of leukemia by industrial hazards rest upon quantitation of the agent and the yield of leukemia. Two agents used in industry have been correlated with an increased incidence of leukemia in human beings. The first, ionizing radiation, is unquestionably able to increase the incidence of leukemia. The second agent, benzol, probably can produce an increased incidence of leukemia, but the data are not as good as for the former. First, the finger of suspicion must be pointed at any agent which is able to produce an aplasia of the bone marrow, assuming it will probably be able to produce leukemia also. Second, there is no reason to doubt that any agent which will produce a cancer elsewhere in the body will not be able to produce leukemia if the offending agent is transported to the hemopoietic tissues.

Benzene fits in the former category because it is well documented that it produces aplasia of the bone marrow.

Current opinion of the leukemogenic potential of benzene can best be summarized by the following investigators' quotations:

> Benzene is the only chemical agent strongly suggested to be leukemogenic on the basis of striking occupational clusters, although recent suspicion has been directed to chloramphenicol.[23]

> Occupational exposure to benzene is generally accepted as incurring a risk of leukemia, especially erythroleukemia, although the incidence of leukemia is low compared with that of hypoplasia affecting one or more of the hemopoietic-cell series.[24]

> Although some authorities still have some doubts, the association between benzene exposure and leukemia is widely accepted as an established fact.[2]

> Even now benzene is the only serious contender for the status of an accepted leukemogen. There is little question that the cases of leukemia--mainly acute--have occurred more frequently in persons with a history of heavy occupational exposure to benzene or its congener toluol than among the general population.[26]

> Since the association between benzene exposure and leukemia was first suggested by Delore and Borgomano, sufficient cases have been reported to provide evidence that benzene can give rise to this disease.[9]

> A cause and effect relationship between benzene and leukemia have been difficult to establish. Despite some negative reports, it now seems fairly clear that chronic exposure to high concentrations of benzene may lead to one of several types of leukemia, the most prevalent of which is acute or subacute myeloblastic leukemia.[60]

The first case of leukemia linked to the action of benzene was described by Delore and Borgomano[15] in 1928. In 1939 Mallory et al.[41] described two cases of leukemia among the patients who had chronic benzene exposures in the industries around Boston. One patient had been exposed to benzene for 10 years, 4 years heavily (200 ppm) and the succeeding 6 years lightly,[10] but had shown hematologic evidence of benzene intoxication from the beginning of his employment.[28] In the later 3 months of his life, the typical pattern of an acute myeloid leukemia developed. The characteristic findings of leukemia were found at autopsy which included diffused myeloid infiltration of the liver, spleen, and bone marrow. The other case was a twelve-year old boy, a painter's son, who used his father's paint shop to repaint toys, and using a paint remover known to contain benzene to remove the preceding coat. He developed aplastic anemia but sternal puncture and sternal biopsy revealed a typical leukemic replacement of the marrow with undifferentiated cells of the lymphoblastic series. Prior to these two cases, 10 cases of leukemia in patients who had been chronically exposed to benzene had been reported.[41] The recently published German document "Benzene in the Work Environment" summarizes over 250 reported cases of leukemia developed in subjects who had been chronically exposed to benzene.[8] The proven cases of leukemia are broken down into three groups: cases of chronic exposure without evidence of acute exposures but without any statement of exposure concentration; cases with only vague exposure data; and cases including a numerical statement of exposure concentrations. This case summary can be interpreted as being indicative of a correlation between chronic benzene exposure and the occurrence of various types of leukemia. These cases have been compiled from individual reports and collective surveys; therefore, it is not possible to estimate the unreported cases or to calculate the incidence of benzene leukemias in the general population, because the total number of persons exposed to benzene cannot be determined. It is also impossible to establish a quantitative relationship between the concentration and duration of exposure and the development of leukemia, because the number of cases with exposure data is too small. In addition, the reliability of the reported benzene concentrations appears very doubtful at best. One case of acute myeloid leukemia was attributed to benzene exposure of less than 25 ppm for over 4 years; however, subsequent studies revealed that prior to the quoted time this subject had been exposed to much higher concentrations of benzene. The authors also suggested that benzene hemopathies are apt to develop in combination with other chemical (or probably physical) effects. This contention is supported by the vast diversity of effects developed in individuals under identical exposure conditions.

DeGowin[14] reported a case involving a painter who had been exposed to benzene for 13 yrs. He developed a hypocellular bone marrow and pancytopenia, followed by a relatively normal bone marrow with variable leukopenia, anemia, and thrombocytopenia. Then after 15 yrs a frankly leukemic

marrow and pancytopenia were found. DeGowin stated, "It is tempting to relate the blood diseases, or disease, to benzene exposure."

In 1971, Girard et al.[25] reported the results of a three-year systematic study conducted in France. The investigation was conducted to determine the exposure to benzene or toluene in 401 subjects with malignant hemopathies and in 124 controls admitted for non-hematologic diseases. The bases of the study were by precise interrogations, inquiries in the work environment, and collection and analysis of products used. The results indicated a much higher frequency of past exposures to benzene or toluene among subjects with aplastic anemia (20.8%), chronic lymphocytic leukemia (14.7%) and acute leukemia (12.1%) than among the control group (4%). These differences were determined to be statistically significant. The use of the hydrocarbons occurred occupationally in 31 cases and domestically in 15 cases. Girard et al.[25] concluded that the action of benzene hydrocarbons in the genesis of malignant hemopathies should not be underevaluated.

In 1972, Aksoy et al.[2] reported four cases of acute leukemia attributed to chronic exposure to benzene. All of these patients had worked in areas which were unhygienic and the concentrations of benzene in air were between 150 and 200 ppm. The occupation of these four patients was shoe manufacturing, and two of the four had suffered from aplastic anemia two and three years respectively, before the onset of acute leukemia. The other two cases of leukemia developed during their exposure to benzene. One developed leukemia without any preceding episode of aplastic anemia and the other developed leukemia followed by a transient episode of pancytopenia lasting approximately two months. However, during the transient period of pancytopenia the bone marrow was suggestive of leukemia which was considered a preleukemic stage. Three of the four cases of leukemia were of the acute myeloblastic type, the most frequent type attributed to chronic exposure to benzene.[4] The clinical and hematologic evaluations of the subjects with acute myeloblastic leukemia due to benzene did not differ from those of patients with acute leukemia not related to benzene exposure. The fourth patient developed thrombocythemia in the second year after an episode of aplastic anemia. At that time the bone marrow did not reveal signs of leukemia, but acute monocytic leukemia developed later. This type of leukemia is rare among patients who develop the disease from benzene exposure.

Aksoy et al.[4] reported that from 1967 to 1973, 26 patients among 28,500 shoeworkers chronically exposed to benzene in Istanbul developed leukemia or preleukemia. The most common diagnosis was acute myeloblastic leukemia, which was seen in 14 of the 26 cases. The duration of benzene exposure varied from 1-15 yrs with a mean of 9.7 yrs. The 26 cases of acute leukemia or preleukemia observed during this 7-yr period were among a group of 28,500 workers at risk, or an annual incidence of 13/100,000. This incidence was reported as statistically significantly greater ($p > 0.02$) than the incidence of 6/100,000 in the general population; however, the statistical methods were not described. A peak incidence of leukemia, 19.7/100,000 occurred between 1971 and 1973 but no additional possible leukemogenic factors could be detected to explain the higher incidence.

The above reported data provide further evidence for an etiologic role for benzene in leukemia; however, the statistical significance of these data should be investigated.

In a recent review article on industrial carcinogens,[18] Eckardt made the following statements:

> The accumulation in the literature of cases of leukemia following benzene exposures leads to the inevitable conclusion that benzene is a leukemogenic agent, particularly in cases that have previously displayed a panmyelopathy. Although this had long been suspected, the data reported in the literature was not sufficiently convincing to establish the leukemogenic nature of benzene. However, the more recent observations seem to establish this association beyond a doubt. More recent observations of chromosome changes in workers exposed to benzene lend further weight to the leukemogenic nature of benzene.

Thorpe[61] conducted the largest epidemiologic survey to date, using workers of eight affiliates of the Exxon Corporation to determine the incidence of leukemia in persons potentially exposed to benzene. Five methodological problems were recognized and discussed which include: low incidence of leukemia in the general population; validity of the diagnosis of leukemia; quantitative determinations of the extent of exposures; inadequate follow-up on annuitants; and incomplete occupational histories on individuals with diagnosed leukemia. Despite the problems and the necessity for making a number of assumptions, Thorpe concluded that the occurrence of leukemia in a population of 38,000 petroleum workers exposed to low levels of benzene for 10 yrs was not different from the general population in the countries concerned.

After Thorpe's paper was published in the Journal of Occupational Medicine, Brown[11] wrote a letter to the editor, which was highly critical of Thorpe's work. Brown concluded his letter with the following statement:

> With case-finding techniques as apparently relaxed as those in the Exxon study by Dr. Thorpe, one cannot help but doubt the accuracy of the data presented.

In 1975 McMichael et al.[42] reported results of an epidemiological study into work-related health problems. The study on rubber industry workers indicates an association between leukemia and jobs entailing exposure to solvents. Xylene, toluene, trichloroethylene and various aliphatic hydrocarbons are among the modern solvents used in the rubber industry. It was determined that the risk of death from lymphatic leukemia appears to be approximately two-fold for workers in the medium or low solvent-exposure jobs (compared to unexposed workers). The data suggest that the leukemogenic agent is a chemical still present, rather than one that was eliminated prior to 1945. The increased incidence is primarily for the lymphatic type as compared to either the hemocytoblastic or myeloblastic types associated with gross benzene intoxication. Data obtained in this study also suggest a possible association between solvent

exposure in the rubber industry and lymphosarcoma, Hodgkin's disease and myeloid leukemia.

Aksoy et al.[5] have reported six cases of Hodgkin's disease in patients with a history of chronic benzene exposure from 1-28 yrs prior to the onset of the disease. A possible etiological relationship between Hodgkin's Disease and benzene is discussed.

Li et al.[40] have conducted a survey to evaluate the cancer experience of chemists. This study was made by examining the causes of death among 3,637 members of the American Chemical Society who died between 1948 and 1967. Malignant lymphomas were significantly increased in male chemists in both the 20-64 year, and over age 64, groups. Leukemia and cancers of the pancreas and intestines were moderately increased in incidence over the controls ($0.01<P<0.1$). The data of this study suggest the influence of carcinogens encountered by chemists in their work place; however, no evaluations could be made as to the type of chemical exposures in relation to causes of death.

As early as 1963, DeGowin suggested, on the basis of the initiation-promotion theory of the induction of neoplasms, that benzene might be an initiator of the leukemia process; however, he had no suggestion of a possible promoter. Ishimaru et al.[31] conducted an epidemiology study to determine if occupational factors contributed to the incidence of leukemia in Hiroshima and Nagasaki. Because benzene and x-rays had been reported to induce leukemia, 11 occupations in which benzene or its derivatives, or x-ray, are frequently involved, were selected for study. The risk of leukemia was found to be about 2.5 times higher among those with a history of such occupations, especially among those who had engaged in these occupations for at least 5 years. Analysis of the data in more detail also revealed that there was an increase in industries where various volatile solvents were used. The data provide no insight as to whether the effects of these agents are additive, synergistic, or may be acting as co-leukemogens.

Aksoy et al.[2] reported two cases of leukemia in patients with aplastic anemia which occurred several years after pancytopenia. These patients had been re-exposed irregularly to benzene after release from the hospital. It was postulated that this irregular re-exposure to benzene may have been a contributing factor in the development of leukemia. In addition, both patients had been treated with corticosteroids for their pancytopenia, which they suggest could have played a role in the development of leukemia.

In addition to earlier attempts to induce neoplasms in mice by benzene, two recent studies have been reported. Laerum[36] found that the occurrence of reticulum cell neoplasms in normal and benzene-treated hairless mice was 39% in each group at a mean age of 20 mos. The benzene-treated mice had been painted twice weekly on the back skin for the duration of the experiment. Ward et al.[67] conducted a study using weanling male C57BL/6N mice, which have been shown to be sensitive to agents such as chemicals and radiation that induce leukemia. The mice received repeated subcutaneous

injections twice weekly for 44 wks and once weekly for the last 10 wks of treatment. After the first 4 wks, the dose was 0.2 ml of 30% benzene in corn oil per injection. The benzene-treated mice and controls were killed 104 wks after the first injection and no evidence of carcinogenic activity was found in either group. Butylnitrosourea, used as a positive control, induced leukemia, lymphomas, and/or intestinal neoplasms in essentially all of the mice.

Since animal experiments do not support the view that benzene is a leukemogen, Ward et al.[67] suggested that benzene-induced leukemia in man may be a fairly rare event occurring only in highly sensitive individuals, because of genetic constitution, or because of synergistic action with other environmental agents. Another point suggested to explain the difference between man and animal models was a difference in metabolism of benzene.

Miller[43] has conducted an epidemiologic study to determine persons with exceptionally high risk of leukemia. He has identified 5 classes of persons whose probability of developing leukemia within a relatively short time is 1 in 100 or greater. The classes represent a surprising diversity of cytogenetic findings; however, a common denominator for each high-risk group is a distinctive genetic or chromosomal feature which may play a role in leukemogenesis. These classes represent a diversity of cytogenetic features, such as genetic identity in the case of identical twin of child with leukemia, genetically-induced chromosomal fragility in Bloom's and Fanconi's syndromes, chromosomal breaks due to radiation, and congenital aneuploidy in Down's syndrome. The genetic feature for each high-risk group may play a role in leukemogenesis. Aksoy et al.[6] have published a report in which acute leukemia has occurred following exposure to benzene in two relatives, a man and his paternal uncle. It was suggested that the two patients with acute leukemia in two generations may have been due to some genetic factors triggered by the chronic exposure to benzene.

Potolsky and Creger,[51] in a recent review article relating radiation and drug therapies and leukemia, have proposed four mechanisms for the induction of leukemia, which are as follows: chromosome breakage; activation of a latent leukemogenic agent (virus?); immunosuppression; and marrow aplasia. These postulated mechanisms are not mutually exclusive and data on benzene clearly indicates that it induces at least three of the effects; however, it must be emphasized that the discovery of the actual mechanisms involved in the etiology of human leukemia awaits further investigations.

RELATIONSHIP BETWEEN BENZENE METABOLISM AND TOXICITY

Because inhalation is the primary route of exposure to benzene, Nomiyama and Nomiyama[45] have investigated the uptake, excretion and respiratory retention of benzene in humans. Six volunteers were exposed to 52-62 ppm benzene for 4 hrs. After 3 hrs exposure, the respiratory retention reached a constant level of 30%. The respiratory uptake at this point was determined to be 46.9% and excretion was 16.8%. Nomiyama and

Nomiyama[46] also studied the respiratory elimination of benzene from humans. Respiratory elimination refers to the excretion after the cessation of the exposure. They found that the concentration of benzene in expired air of persons exposed to benzene decreased from 10 ppm to 0.5 ppm in 17 hours after exposure. Significant amounts of benzene were eliminated unchanged through the lungs. Hunter and Blair[29] have found that humans retain approximately 230 mg of benzene from exposure to 80-100 µg/l for 6 hrs. It was determined that the elimination via the lungs is no more than 12% of the retained dose. This value is significantly less than elimination losses found in animals which are normally quoted in the range 50-70%. Phenol measurement (free plus combined) on the urine of the human volunteers indicated that 50-87% of the retained benzene was excreted as phenol. The highest concentration of phenol was found in the urine within about 3 hrs from the termination of the exposure.

In addition to phenol, hydroquinone, catechol, hydroxyhydroquinone, trans-transmuconic acid and L-phenylmercapturic acid are excreted in the urine.[60] The phenolic metabolites are found primarily as glucuronide conjugates and ethereal sulfate which can be liberated by hydrolysis in strong acid. The metabolic fate of ^{14}C-phenol has been studied in 19 species including man. The major route of metabolism in all species was conjugation which included both ethereal sulfate and glucuronide conjugates, except for the pig and the cat. The cat excreted the total dose as phenylsulfate (87%) and hydroquinone sulfate (13%) while the pig excreted only phenylglucuronide.

The rate of benzene metabolism depends on the dose given and the presence of compounds which either stimulate or inhibit benzene metabolism. Pretreatment with phenobarbital has been reported to increase the rate of metabolism in both the rat and mouse.

Snyder and Kocsis[60] have also reviewed the in vitro studies on benzene metabolism. Evidence taken from several sources collectively indicate that benzene is hydroxylated by cytochrome P450 and the mixed function oxidase. The mechanism of benzene hydroxylation has not been determined; however, it has been suggested that the reactions probably occur via an arene oxide intermediate. Benzene oxide has not been found in liver microsomes, probably due to its extreme lability, but naphthalene oxide has been found when naphthalene was incubated with microsomes. In addition, the incubation of benzene oxide with microsomes yields the metabolic products of benzene.

The review of Nomiyama et al. work[60] noted that young rats displaying a high rate of benzene metabolism in vitro were also more susceptible to subcutaneous injections of benzene than older rats exhibiting slower benzene metabolism. They also reported protection against benzene toxicity by 3-amino-1,2,4-triazole, which inhibits benzene metabolism in liver homogenates. It was thus concluded that a metabolic product of benzene was responsible for its hemotoxic effects. Ikeda and Ohtsuji[30] treated rats with phenobarbital and found a 3- to 4-fold increase in aromatic hydroxylase measure in vitro. In vivo studies with phenobarbital pretreated rats showed an enhanced excretion of phenol in the urine. This

suggests increased benzene metabolism since phenol is the major metabolite of benzene. When phenobarbital pretreated rats were exposed to benzene vapors an increased tolerance to the leukopenic action of benzene was observed. These data were interpreted to support the idea that benzene itself is the actual toxic substance. Saito et al.[54] reported that benzene metabolism is increased in rat liver microsomes after treatment of rats with benzene, but not by treatment with resorcinol or phenol; however, treatment with hydroquinone, pyrogallol or pyrocatechol actually decreased the in vitro metabolism of benzene.

Drew and Fouts[17] have shown that both phenobarbital and 3-methylcholanthrene induced benzene hydroxylase in rat liver; however, only chlorpromazine induced benzene hydroxylase in the lung, and this was only minimal induction. The LC_{50} for inhaled benzene and the LD_{50} for injected benzene were not changed by pretreatment of rats with either phenobarbital, 3-methylcholanthrene or chlorpromazine. It was emphasized that while the induction of metabolizing systems may not alter acute toxicity, they may play a more important role in chemical toxicity due to chronic exposures.

Lee et al.[39] have used the erythrocyte ^{59}Fe utilization method to study the effect of benzene on erythropoiesis in mice. After benzene administration in doses of 440 or 2200 mg/kg, bone marrow suppression was first detected 24 hours post-treatment with maximum suppression after 48 hours and complete recovery noted after 72 hours. The evidence in this study suggests that benzene inhibits the multiplication of the erythrocyte precursor cells and does not affect the incorporation of iron into heme.

Snyder and Kocsis,[60] in their review, reported some additional results from their laboratory using the ^{59}Fe utilization method and labeled benzene. They found that when mice were administered toluene, a competitive inhibitor of benzene metabolism, the rate of benzene metabolism was reduced, and the ^{59}Fe uptake into erythrocytes increased, thus indicating that benzene toxicity was alleviated. When mice were pretreated with phenobarbital to increase the mixed function oxidase activity, an increase in benzene metabolism occurred with a concurrent decrease in ^{59}Fe uptake. The results of these studies support the view that a metabolic step is important in causing benzene toxicity.

Mitchell[44] has reported that phenol, catechol, and hydroquinone administered to rats for 1 wk failed to induce aplastic anemia, although the highest doses killed approximately 50% of the animals. It was postulated that the active metabolite was neither phenol, catechol or hydroquinone as previously thought.

The above studies suggest that benzene metabolism may be of considerable importance in benzene toxicity; however, the evidence is not definitive. A problem pointed out by Snyder and Kocsis[60] is that the studies conducted on the metabolism of benzene, whether in vivo or in vitro, have been conducted in the liver. Chronic benzene toxicity manifests itself in the bone marrow, thus the active metabolite must either be transported from the liver to the bone marrow or the metabolite is produced in in the bone marrow. Since it has been suggested that RNA and DNA synthesis

may influence benzene toxicity, benzene oxide is a highly reactive species that would be an excellent candidate for disturbing DNA and RNA synthesis. The reactivity of benzene oxide thus would preclude its transport from the liver to the bone marrow. It would appear that studies on benzene metabolism in bone marrow would be best for elucidating of the mechanism of benzene toxicity. This is emphasized by the statement of Snyder and Kocsis:

> In any event, a point has been reached in the study of benzene toxicity where we must recognize that there can be no more direct approach to studying the disease than to investigate it in the organ where it occurs.

REFERENCES

1. Aksoy, M., K. Dinçol, T. Akgün, S. Erdem, and G. Dinçol. 1971. Haematological effects of chronic benzene poisoning in 217 workers. Brit.J.Ind.Med. 28:296-302

2. Aksoy, M., K. Dinçol, S.Erdem, and G. Dinçol. 1972. Acute leukemia due to chronic exposure to benzene. Am.J.Med. 52:160-166.

3. Aksoy,M., K.Dinçol, S.Erdem, T. Akgün, and G. Dinçol. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Brit.J.Ind.Med. 29:56-64.

4. Aksoy, M., S. Erdem, and G. Dinçol. 1974. Leukemia in shoe-workers exposed chronically to benzene. Blood 44:837-841.

5. Aksoy, M., S. Erdem, K. Dinçol, T. Hepyüksel, and G. Dincol. 1974. Chronic exposure to benzene as a possible contributary etiologic factor in Hodgkin's disease. Blut 28:293-298.

6. Aksoy, M., S. Erdem, G. Erdogan, and G. Dinçol. 1974. Acute leukaemia in two generations following chronic exposure to benzene. Hum.Hered. 24:70-74.

7. American National Standards Institute, Inc. 1969. Acceptable concentrations of benzene. Z37.4-1969. New York, ANSI.

8. Benzene in the Work Environment. Considerations Bearing on the Question of Safe Concentrations of Benzene in the Work Environment (MAK-Wert). 1974. Communication of the Working Group "Establishment of MAK-Werte" of the Senate Commission for the Examination of Hazardous Industrial Materials, prepared in cooperation with Dr. Gertrud Buttner. Boppard, Germany, Harald Boldt Verlag.

9. Berlin, M., J. Gage, and E. Johnson. 1974. Increased aromatics in motor fuels: A review of the environmental and health effects. Work Environ. Health 11:1-20.

10. Bowditch, M., and H.B. Elkins. 1939. Chronic exposure to benzene (benzol). I. The industrial aspects. J.Ind.Hyg.Toxicol. 21:321-330.

11. Brown, S.M. 1975. Leukemia and potential benzene exposure. J. Occup. Med. 17:5-6.

12. Browning, E. 1965. Toxicity and Metabolism of Industrial Solvents. New York, Elsevier.

13. Cronkite, E.P. 1961. Evidence for radiation and chemicals as leukemogenic agents. Arch.Environ.Health 3:297-303.

14. DeGowin, R.L. 1963. Benzene exposure and aplastic anemia followed by leukemia 15 years later. J.Am.Med.Assoc. 185:748-751.

15. Delore, P., and Borgomano. 1928. Leucémie aiguë au cours de l'intoxication benzénique. Sur l'origine toxique de certaines leucémies aiguës et leurs relations avec les anémies graves. J.Med.Lyon 9:227-233.

16. Dobrokhotov, V.B. The mutagenic influence of benzene and toluene under experimental conditions. Gig.Sanit. 37(10):36-39 1972. [Translated by Air Pollution Technical Information Center, Environmental Protection Agency, Research Triangle Park, N.C. Translation No. HS-138.]

17. Drew, R.T., and J.R. Fouts. 1974. The lack of effects of pre-treatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol.Appl.Pharmacol.27:183-193.

18. Eckardt, R.E. 1973. Recent developments in industrial carcinogens. J.Occup.Med. 15:904-907.

19. Forni, A.M., A. Cappellini, E. Pacifico, and E. C. Vigliani. 1971. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch.Environ.Health 23:385-391.

20. Forni, A., and L. Moreo. 1967. Cytogenetic studies in a case of benzene leukaemia. Eur.J.Cancer 3:251-255.

21. Forni, A., and L.Moreo. 1969. Chromosome studies in a case of benzene-induced erythroleukaemia. Eur.J.Cancer 5:459-463.

22. Forni, A., E. Pacifico, and A. Limonta. 1971 Chromosome studies in workers exposed to benzene or toluene or both. Arch.Environ. Health 22:373-378.

23. Fraumeni, J.F., Jr. 1969. Clinical epidemiology of leukemia. Semin.Hematol.6:250-260.

24. Galton, D.A.G., and A.S.E. Spiers. 1971. Progress in the leukemias. Prog.Hematol. 7:343-405.

25. Girard, R., F. Tolot, and J. Bourret. 1971. Malignant haemopathies and benzene poisoning. Med.Lav. 62:71-76.

26. Gunz, F., and A.G. Baikie. 1974. Leukemia, 3rd edition. New York, Grune and Stratton.

27. Hartwich, G., G. Schwanitz, and J. Becker. 1969. Chromosome anomalies in a case of benzene leukaemia. Ger.Med. Monthly 14:449-450.

28. Hunter, F.T. 1939. Chronic exposure to benzene (benzol). II. The clinical effects. J.Ind.Hyg.Toxicol. 21:331-354.

29. Hunter, C.G., and D. Blair. 1972. Benzene: Pharmacokinetic studies in man. Ann. Occup.Hyg. 15:193-199.

30. Ikeda, M., and H. Ohtsuji. 1971. Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. Toxicol. Appl. Pharmacol. 20:30-43.

31. Ishimaru, T., H. Okada, T. Tomiyasu, T. Tsuchimoto, T. Hoshino, and M. Ichimaru. 1971. Occupational factors in the epidemiology of leukemia in Hiroshima and Nagasaki. Am.J.Epidemiol. 93:157-165.

32. Khan, H., and M.H. Khan. 1973. Cytogenetic studies following chronic exposure to benzene. Arch.Toxikol. 31:39-49.

33. Kissling, M., and B.Speck. 1971. Chromosome aberrations in experimental benzene intoxication. Helv.Med.Acta 36:59-66.

34. Kissling, M. and B. Speck. 1972. Further studies on experimental benzene induced aplastic anemia. Blut 25:97-103.

35. Koizumi, A., Y. Dobashi, Y. Tachibana, K. Tsuda, and H. Katsunuma. 1974. Cytokinetic and cytogenetic changes in cultured human leucocytes and HeLa cells induced by benzene. Ind.Health (Japan) 12:23-29.

36. Laerum, O.D. 1973. Reticulum cell neoplasms in normal and benzene treated hairless mice. Acta Pathol.Microbiol.Scand.Sect.A 81:57-63.

37. Lange, A., R. Smolik, W. Zatonski, and J. Szymanska. 1973. Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. Int. Arch. Arbeitsmed. 31:37-44.

38. Lange, A., R. Smolik, W. Zatonski, and H. Glazman. 1973. Leukocyte agglutinins in workers exposed to benzene, toluene and xylene. Int.Arch.Arbeitsmed. 31:45-50.

39. Lee, E.W., J.J. Kocsis, and R. Snyder. 1974. Acute effect of benzene on ^{59}Fe incorporation into circulating erythrocytes. Toxicol.Appl. Pharmacol. 27:431-436.

40. Li, F.P., J.F. Fraumeni, Jr., N. Mantel, and R. W. Miller. 1969. Cancer mortality among chemists. J.Natl.Cancer Inst. 43:1159-1164.

41. Mallory, T.B., E.A. Gall, and W.J. Brickley. 1939. Chronic exposure to benzene (benzol). III. The pathologic results. J. Ind.Hyg.Toxicol. 21:355-393.

42. McMichael, A.J., R. Spirtas, L.L. Kupper, and J.F. Gamble. 1975. Solvent exposure and leukemia among rubber workers: An epidemiologic study. J.Occup.Med. 17:234-239.

43. Miller, R.W. 1967. Persons with exceptionally high risk of leukemia. Cancer Res. 27:2420-2423.

44. Mitchell, J.R. 1972. Mechanism of benzene-induced aplastic anemia. Fed.Proc.Fed.Am.Soc.Exp.Biol. 30:561Abs, #2044.

45. Nomiyama, K., and H. Nomiyama. 1974. Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int.Arch.Arbeitsmed. 32:75-83.

46. Nomiyama, K., and H. Nomiyama. 1974. Respiratory elimination organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int.Arch. Arbeitsmed. 32:85-91.

47. Parkinson, G.S. 1971. Benzene in motor gasoline--an investigation into possible health hazards in and around filling stations and in normal transport operations. Ann. Occup.Hyg. 14:145-153.

48. Pierre, R.V. 1974. Preleukemic states. Semin.Hematol. 11:73-92.

49. Pollini, G., and R. Colombi. 1964. Lymphoctye chromosome damage in benzene blood dyscrasia. Med.Lav.55·641-654.

50. Pollini, G., E. Strosselli, and R. Colombi. 1964. Relationship between chromosomal alterations and severity of benzol blood dyscrasia. Med.Lav. 55:735-751.

51. Potolsky, A., and W.P. Creger. 1973. Radiation and drug therapies, and leukemia. Annu.Rev.Med. 24:75-82.

52. Runion, H. 1975. Benzene in gasoline. J.,Am.Ind.Hyg.Assoc. 36:338-350.

53. Saita, G. 1973. Benzene induced hypoplastic anaemias and leukaemias. IN Girdwood, R.H., ed. Blood Disorders Due to Drugs and Other Agents. Amsterdam, Excerpta Medica. 1973. p. 127-146.

54. Saito, F.U., J.J. Kocsis, and R. Snyder. 1973. Effect of benzene on hepatic drug metabolism and ultrastructure. Toxicol. Appl. Pharmacol.26:209-217.

55. Sandberg, A.A. 1966. The chromosomes and causation of human cancer and leukemia. Cancer Res. 26:2064-2081.

56. Selling, L. 1916. Benzol as a leucotoxin. Studies on the degeneration and regeneration of the blood and haematopoietic organs. John Hopkins Hosp.Reports. 17:83-142.

57. Sellyei, M., and E. Kelemen. 1971. Chromosome study in a case of granulocytic leukaemia with 'Pelgerisation' 7 years after benzene pancytopenia. Eur.J.Cancer 7:83-85.

58. Sherwood, R.J. 1972. Evaluation of exposure to benzene vapour during the loading of petrol. Brit.J.Ind.Med. 29:65-69.

59. Smolik, R., K. Grzybek-Hryncewicz, A.Lange, and W. Zatonski. 1973. Serum complement level in workers exposed to benzene, toluene and xylene. Int.Arch.Arbeitsmed. 31:243-247.

60. Snyder, R., and J.J. Kocsis. 1975. Current concepts of chronic benzene toxicity. CRC Crit.Rev.Toxicol. 3:265-288.

61. Thorpe, J.J. 1974. Epidemiologic survey of leukemia in persons potentially exposed to benzene. J.Occup.Med. 16:375-382.

62. TLVs®. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1975. 1975. Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists.

63. Tough, I.M., and W.M. Court Brown. 1965. Chromosome aberrations and exposure to ambient benzene. Lancet 1:684.

64. Tough, I.M., P.G. Smith, W.M. Court Brown, and D.G. Harnden. 1970 Chromosome studies on workers exposed to atmospheric benzene. The possible influence of age. Eur.J.Cancer 6:49-55.

65. U. S. Department of Health, Education, and Welfare. Public Health Service, National Institute for Occupational Safety and Health. 1974. Criteria for a Recommended Standard .... Occupational Exposure to Benzene. HEW Publ.No. (NIOSH) 74-137. Washington, D.C., U.S. Government Printing Office.

66. Vigliani, E.C., and A. Forni. 1969. Benzene, chromosome changes and leukemia. J.Occup.Med. 11:148-149.

67. Ward, J.M., J.H. Weisburger, R.S. Yamamoto, T. Benjamin, C.A. Brown, and E.K. Weisburger. 1975. Long-term effect of benzene in C57BL/6N mice. Arch.Environ.Health 30:22-25.

68. Yakushevich, Yu.Ye. 1973. Experimental data on the hygienic evaluation of continuous and interrupted action of benzene toluene and xylene. Gig.Sanit. 38(4):6-9 1973. [Translated by Air Pollution Technical Information Center, Environmental Protection Agency, Research Triangle Park, N.C. Translation No. HS-135.]